Summer Polysaccharide Content in Seven Species of West Coast Intertidal Prosobranch Snails

BY

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(Plate 11)

INTRODUCTION

MANY MEASUREMENTS of the glycogen content of mollusks have been made (review by MARTIN, 1961) but there are few reports in the literature on marine snails. West Coast species have received little attention, and no comparative studies have been made on snails with different diets.

In snails, the study of glycogen is complicated by the occurrence of galactogen. Galactogen, a polymer of galactose units, occurs exclusively in the albumen glands and the eggs of several species of terrestrial and fresh-water snails, and in two bivalves (reviews by MARTIN, 1961; and GODDARD *et al.*, 1963). MCMAHON *et al.* (1957) found a galactose-fucose polysaccharide associated with two species of operculate snails. They also studied some snails with separate sexes and found that galactogen was present only in the albumen gland and eggs. Glycogen was the only polysaccharide present in males.

BARRY & MUNDAY (1959), however, found only glycogen throughout the year in *Patella vulgata*, a marine prosobranch. A large quantity of ether extractable fat seemed to be the major reserve material in the sex products.

This paper presents a comparative survey of the amount of polysaccharides occurring in seven species of marine prosobranch snails during the summer months, and an identification of the sugars occurring in the acid hydrolysates of these polysaccharides.

MATERIALS AND METHODS

Tegula funebralis (A. ADAMS, 1854), *Calliostoma ligatum* (GOULD, 1846), *Littorina scutulata* (GOULD, 1849), *Searlesia dira* (REEVE, 1846), *Thais emarginata* (DESHAYES, 1839), *Thais lamellosa* (GMELIN, 1792), and *Olivella biplicata* (SOWERBY, 1825) were studied. All snails were collected at low tides during July and August, 1964. *Olivella biplicata* were taken from a sandy beach near the Oregon Institute of Marine Biology station, Charleston, Oregon. The other six species were collected from rocks at Point Arago, Coos County, Oregon.

The study was started at the Oregon Institute of Marine Biology on fresh material, and completed on frozen material at the Laboratories of Zoöphysiology, University of South Dakota. When fresh snails were used, dry weights were calculated from the data given in table 1. When frozen snails were used, the soft parts were separated from the shell and dried in a vacuum dessicator over $CaCl_2$.

Measurement of total polysaccharides: Polysaccharides were precipitated from 30% KOH hydrolysates of whole snails and estimated as glucose after hydrolysis in 1N HCl (BARNES, *et al.* 1963).

Chromatographic identification of sugars: Polysaccharides for chromatography and electrophoresis were precipitated from 30% KOH and purified by repeated reprecipitations with ethanol. Samples of this purified polysaccharide weighing 10 mg were hydrolyzed in 1.0 ml of 1N H_2SO_4 for 3 hours at 100° C and neutralized with solid barium carbonate. After removal of the barium sulfate precipitate by centrifugation, samples of the supernate were spotted on Whatman no. 1 chromatographic paper (MCMAHON, *et al.* 1957). One dimensional descending chromatography was used, the solvent being allowed to drip from the serrated lower end of the paper. Development for 36 - 40 hours separated the sugars adequately for identification. Two solvent systems were used: butanol, ethanol, water (4:1:5) (PARTRIDGE, 1946) and butanol, acetic acid, water (4:1:5) (PARTRIDGE, 1948). The butanol, ethanol, water (4:1:5) gave superior results. Sugars were identified with ammoniacal silver nitrate (TREVELYAN, *et al.* 1950) and aniline-oxalate (CLARK,

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1964). A 0.1% solution of ninhydrin in 1-butanol was used to detect amino sugars (Hough & Jones, 1962). Sugars on chromatograms of *Calliostoma ligatum* were quantitated by densitometer readings (Photovolt Corporation, Model 52-C). Known sugars were used as standards.

Electrophoretic identification of sugars: Identification of sugars was confirmed on an LKB 3276B paper electrophoresis unit. Portions of polysaccharide hydrolysates were stripped on LKB-2043B filter paper strips. Good separation of sugars was obtained by applying 20 volts/cm for 4 hours in a 1% solution of borax (pH = 9.2). Sugars were identified by spraying with aniline-oxalate to which a few drops of glacial acetic acid were added to overcome the alkalinity of the borate buffer.

Electrophoretic migration of polysaccharides: Electrophoretic migration of snail polysaccharides before HCl hydrolysis was compared with that of known glycogen. Polysaccharides (10 mg) were dissolved in 0.1 ml water, stripped on LKB-2043B filter paper strips and subjected to 10.3 volts/cm for 6 hours in the 1% borax. The paper strips were removed from the cell, and the polysaccharides were fixed and stained by the periodate-Schiff method of Köiw & Grönwall (1952).

Lipid determinations: Total lipids of *Tegula funebralis* were weighed after evaporation of the ether extracts of whole snails hydrolyzed in 30% NaOH (von Brand *et al.* 1957).

RESULTS

Table 1 summarizes the relative weights of shell and soft tissue used in calculating dry weights from fresh snails.

Table 2 summarizes the total polysaccharide content in the 7 species, and the sugars found in the hydrolysates. Polysaccharides are expressed as percent of dry weight of soft parts. Figures 1 and 2, Plate 11, show typical chromatographic and electrophoretic separation of sugars. No amino sugars were detected by the ninhydrin spray. The hydrolysates of all snails except *Calliostoma ligatum* contained only glucose. The polysaccharide material from this species showed glucose, galactose and ribose. Chromatograms of material from males and from females were similar in their sugar content.

Figure 3 shows typical electrophoretic migration of snail polysaccharide compared with known glycogen. The migration of material from all 7 species, including *Calliostoma ligatum* is identical with that of glycogen. There is no apparent separation of *C. ligatum* polysaccharides.

Table 1

Relative weights of shell and soft tissues of seven species of marine Prosobranch snails. The figures behind the ± signs give confidence limits at the 95% level. The figures in parentheses indicate the number of determinations.

Species	average fresh wt. (grams)[1]	shell wt. as % of fresh wt.	% dry wt. of soft parts as % of fresh wt.	% dry material of soft parts
Littorina scutulata (24)	0.77 ± 0.02	76.7 ± 0.41	4.7 ± 0.33	23.5 ± 1.51
Tegula funebralis (25)	2.95 ± 0.62	68.7 ± 0.56	9.7 ± 0.27	35.0 ± 0.62
Calliostoma ligatum (25)	1.23 ± 0.06	69.9 ± 0.64	7.9 ± 0.28	29.5 ± 0.57
Olivella biplicata (24)	1.60 ± 0.33	69.0 ± 0.13	8.3 ± 0.41	33.7 ± 1.23
Searlesia dira (15)	5.93 ± 0.93	71.0 ± 0.96	8.6 ± 0.64	26.4 ± 1.44
Thais emarginata (20)	0.93 ± 0.15	62.3 ± 0.96	9.3 ± 0.44	31.3 ± 1.75
Thais lamellosa (10)	10.30 ± 2.50	78.7 ± 2.01	4.5 ± 0.86	28.0 ± 3.44

[1] Fresh weight was determined after the outside surfaces of the shells were dry. The operculum was forced back and excess water was removed by blotting with a piece of filter paper.

Table 2

Total polysaccharide content and sugars present in hydrolysates of polysaccharides of seven species of marine Prosobranch snails. The figures behind the ± signs give confidence limits at the 95% level. The figures in parentheses indicate the number of determinations.

Species	% polysaccharide of dry weight (♀ ♀)	% polysaccharide of dry weight (♂ ♂)	% polysaccharide of dry weight (♂ ♂ and ♀ ♀ combined)	% sugars found in hydrolysates of polysaccharides
Thais lamellosa	5.80 ± 1.59 (25)	6.63 ± 1.48 (11)	6.05 ± 1.16 (36)	glucose[1], 100%
Searlesia dira	5.20 ± 0.96 (28)	4.38 ± 1.34 (20)	5.01 ± 0.76 (48)	glucose[1], 100%
Littorina scutulata	.....	.....	3.19 ± 0.30 (59)	glucose[2], 100%
Olivella biplicata	3.37 ± 0.23 (19)	2.66 ± 0.42 (13)	3.14 ± 0.38 (32)	glucose[1], 100%
Thais emarginata	.....	.....	2.90 ± 0.43 (48)	glucose[2], 100%
Tegula funebralis	1.84 ± 0.18 (33)	2.04 ± 0.24 (28)	1.93 ± 0.16 (61)	glucose[3], 100%
Calliostoma ligatum	1.63 ± 0.23 (28)	1.19 ± 0.17 (25)	1.42 ± 0.15 (53)	glucose[3], 55.0 ± 5.6% ribose, 25.6 ± 6.0% galactose 23.3 ± 6.0%

[1] Male visceral mass and foot, and female visceral mass (with eggs) and foot were examined
[2] Combined whole male-female polysaccharides were examined
[3] Whole males and females were examined separately. The values reported for *Calliostoma ligatum* represent combined male- female polysaccharides

Table 3 summarizes the total ether extractable material of *Tegula funebralis.*

Table 3

Total ether extractable material of *Tegula funebralis.* The figures behind the ± signs give confidence limits at the 95% level. The figures in parentheses indicate the number of determinations.

Tissue examined	Female	Male
Visceral mass	12.1 ± 4.7 (4)	2.3 ± 0.9 (4)
Foot	4.3 ± 2.4 (4)	5.5 ± 4.2 (4)
Total	13.9 ± 4.5 (5)	7.1 ± 3.8 (5)

DISCUSSION

Calliostoma ligatum was the only species in which the polysaccharide contained sugars other than glucose (Table 2; figures 1 and 2). In this species, the polysaccharide material was composed of glucose, galactose, and ribose in both sexes. All other species contained only glucose (Table 2). These results indicate that glycogen is the major polysaccharide in the marine forms studied, and that galactogen is not associated with female sex products. In *Tegula funebralis,* much ether extractable material is associated with the gravid female gonad (Table 3). These observations agree with those of Barry & Munday, 1959, who found fat, rather than galactogen associated with the sex products of *Patella vulgata.* The absence of

Explanation of Plate 11

Figure 1

Typical descending chromatographic separation of sugars in butanol, ethanol, water (4:1:5) for 40 hours. All snail hydrolysates are combined male-female samples. – 1. *Thais lamellosa;* 2. *Searlesia dira;* 3. *Thais emarginata;* 4. *Calliostoma ligatum;* 5.(a) galactose, (b) glucose, (c) ribose; 6. galactose; 7. glucose; 8. *Tegula funebralis;* 9. *Littorina scutulata;* 10. *Olivella biplicata*

Figure 2

Typical electrophoretic separation of sugars (20 volts/cm for 4 hours in a 1% borax solution). 1. glucose; 2.(a) ribose, (b) galactose, (c) glucose; 3. galactose

Figure 3

Typical electrophoretic migration of polysaccharides (10.3 volts/cm for 6 hours in 1% borax). 1. glycogen; 2. *Tegula funebralis* ♀; 3. *Thais lamellosa,* visceral mass, ♀; 4. *Calliostoma ligatum*-glycogen mixture

Figure 1

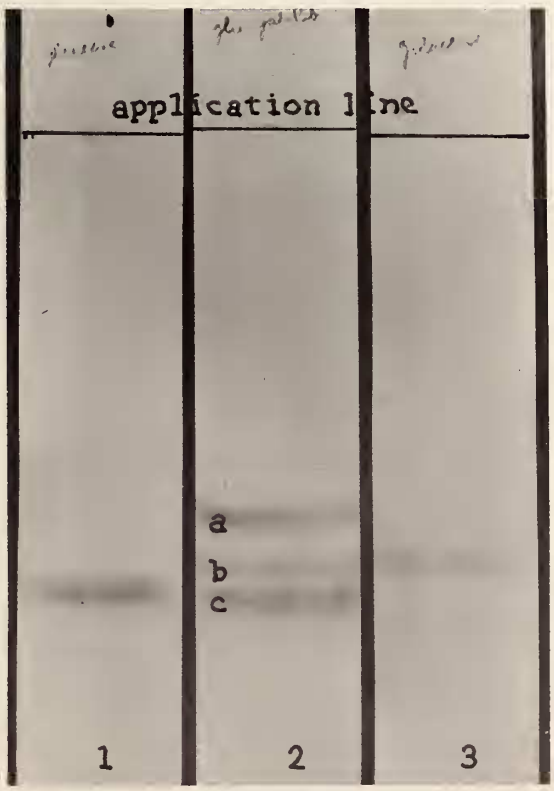

Figure 2

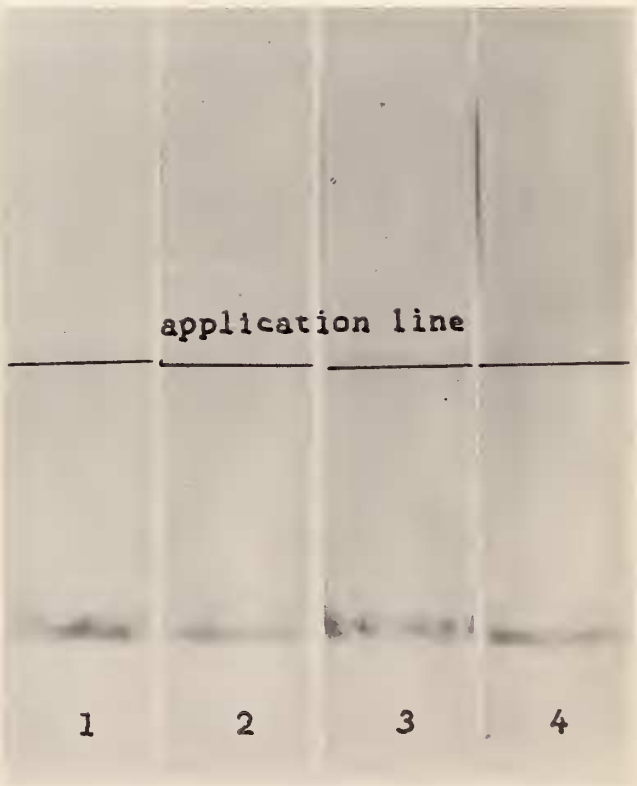

Figure 3